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Date: August 19, 1997 By: Jan Huss

Patent
Attorney's Docket No. 010055-134

#14 D.9 9/3/97

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
SIMON C. BURTON, et al.	Group Art Unit: 1808
Application No.: 08/468,610	Examiner: Jon P. Weber, Ph.D.
Filed: June 6, 1995	
For: CHROMATOGRAPHIC RESINS AND METHODS FOR USING SAME	

BRIEF FOR APPELLANT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This appeal is from the decision of the Primary Examiner dated August 19, 1996 finally rejecting claims 1-5 and 7-23, which are reproduced as Appendix I to this brief. A Notice of Appeal from this final rejection was filed for this application on February 24, 1997 and a four-month extension of time to file this Appeal Brief accompanies this response.

A check covering the requisite Government fee for filing this appeal and the four-month extension of time is submitted herewith as well as two extra copies of this brief as required by 37 C.F.R. §1.192(a)

The Commissioner is authorized to charge any fees that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800.

I. Real Party in Interest

The real party in interest in this application is Massey University assignee of the entire right, title and interest to this application by virtue of an assignment in the parent application as well as Genencor International, Inc., a licensee of Massey University under this application.

II. Related Appeals and Interferences

There are no other appeals and/or interferences related to the appeal taken in this application.

III. Status of Claims

Claims 1-5 and 7-23 remain in this application with Claims 6 and 24-54 having been cancelled.

Claims 1-5 and 7-23 stand finally rejected under 35 USC § 103 as being unpatentable over Sasaki, et al., *J. Biochem.*, 86:1537-1548 (1979) ("Sasaki '79") or Sasaki, et al., *J. Biochem.*, 91:1551-1561 (1982) (hereinafter "Sasaki '82") in view of Kasche, et al., *J. Chromatogr.*, 510:149-154 (1990) (hereinafter "Kasche"), Teichberg, *J. Chromatogr.*, 510:49-57 (1990) (hereinafter "Teichberg") and Jost, et al., *Biochem. Biophys. Acta*, 362:75-82 (1974) (hereinafter "Jost").

IV. Status of Amendments

Subsequent to the final Office Action of August 19, 1996, amendment of Claims 1 and 16 was requested in Appellants' response filed pursuant to 37 C.F.R. 1.116(a) on February 24, 1997. In the Advisory Action mailed March 13, 1997, Appellants were advised that this amendment will be entered into the application upon filing of an Appeal

Brief. No other amendments have been requested for entry into this application subsequent to the final Office Action. Accordingly, the listing of the claims on appeal as set forth in the attached Appendix reflect the amendments requested with the Response to the Final Office Action.

V. Summary of the Invention

The presently claimed invention is directed to a composition comprising a resin-protein complex[1]. In one embodiment, this complex comprises a resin and a protein or peptide bound thereto wherein the resin comprises a solid support matrix and selected ionizable ligand covalently attached to the matrix. The ionizable ligand is selected such that (1) the resin is electrostatically uncharged at the pH where the protein or peptide binds to the resin and the protein or peptide binds to the resin at a pH of 5 to 9; and (2) the resin is electrostatically charged at the pH where the protein or peptide is desorbed from the resin wherein desorption occurs by a change in the pH from the binding pH. The resin is further characterized by the fact that about 50 percent or more of the protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or low ionic strength.

In another embodiment, the resin-protein/peptide complex of this invention comprises a resin and a protein or peptide bound thereto wherein the resin comprises a solid support matrix having a selected ionizable functionality incorporated into the backbone thereof. In this case, the ionizable functionality is selected such that: (1) the resin is electrostatically uncharged at the pH where the protein or peptide is bound to the resin wherein the protein or peptide binds to the resin at a pH of 5 to 9; and (2) the resin is electrostatically charged at the pH where the protein or peptide is desorbed from the

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[1] The term "protein" in resin/protein complexes is intended to cover both proteins and peptides. Moreover, as noted during the interview, Applicants no longer rely upon the Becker declaration as it relates to either Sasaki article and this declaration should be disregarded.

resin wherein desorption occurs by a change in the pH from the binding pH. The resin is further characterized by the fact that about 50 percent or more of the protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or low ionic strength. In an optional aspect of this embodiment, such resins contain a non-ionizable ligand covalently attached thereto.

The specification and claims describe, in detail, such compositions at from page 7, line 25, to page 8, line 9, which describe all of the salient features of the resins of the first embodiment except for the pH range of binding the protein/peptide to the resin which is recited at page 29, lines 24-28, and pH change for desorption which is recited at page 28, lines 22 et seq, as well as in Example X(A) (which illustrates binding at pH 9.1 and desorption at pH 5.2) and Example X(B) (which illustrates binding at pH 7.7 and desorption at pH 5.2). Similarly, the salient features of the resins of the second embodiment are recited at page 8, lines 10-22, at page 55, in original Claim 16 and at page 29, lines 24-28, as well as at page 28, lines 22 et seq and Examples X(A & B).

VI. The Issues

The sole issue for consideration by the Board of Appeals and Patent Interferences is as follows:

is the rejection of Claims 1-5 and 7-23 under 37. U.S.C. §103 over Sasaki '79 or Sasaki '82 in view of Kasche, Teichberg and Jost proper?

VII. Grouping of Claims

All of the appealed claims, namely 1-5 and 7-23, will be grouped together for the purpose of Appellants' argument.

VIII. Argument

Background

In order to facilitate understanding of Appellants' arguments for the above issues, Appellants will first discuss the basis of the present invention in more detail than that set forth in Paragraph V above.

Appellants' claimed invention is directed to a resin/protein complex which, accordingly, infers that the claims covering this invention are composition claims. In these complexes, the resin is characterized, in part, as being electrostatically uncharged when the protein is bound to the resin at a pH of from 5 to 9 and is further characterized as being electrostatically charged at the pH of desorption. Still further, Appellants' claimed invention recites that the pH where the protein or peptide is bound to the resin is different than the pH where the protein or peptide is desorbed from the resin.

Among other factors, Appellants' claimed invention is based on the discovery that resin-protein complexes wherein the resin is electrostatically uncharged at the pH where the protein or peptide is bound to the resin provides, in part, for an efficient binding of the protein to the resin for example from an aqueous media having either high or low ionic strength. Moreover, a binding pH of from 5 to 9 provides for resin/protein complexes which avoid the use of strong acidic/basic conditions which can denature some proteins. See, for example, page 29, lines 24 et seq., of Appellants' specification.

Moreover, as to the resins described in Appellants' first embodiment and recited in Claims 1-5 and 7-15, the ionizable functionality which is uncharged at the pH of protein/peptide binding and charged at the pH of protein/peptide desorption is the ionizable ligand covalently attached to the resin.

As to the resins described in Appellants' second embodiment and recited in Claims 16-23, the ionizable functionality which is uncharged at the pH of protein/peptide binding and charged at the pH of protein/peptide desorption is an ionizable functionality incorporated into the backbone of the resin.

General Argument as to Claims 1-5 and 7-23

Appellants maintain, for the reasons noted below, that the rejection of Claims 1-5 and 7-23 under 35 U.S.C. §103 over Sasaki '79 or Sasaki '82 in view of Kasche, Teichberg and Jost is in error because there is simply no motivation to combine the cited references in the manner of the claimed invention.

Initially, Appellants note that the test for non-obviousness articulated by the Court of Appeals for the Federal Circuit in *In re Vaeck* requires consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition; and (2) whether the prior art would also have provided a reasonable expectation of success to such a skilled artisan. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

Thus, in order to establish a *prima facie* case of obviousness the USPTO must show some objective teaching in the prior art or that knowledge generally available to one skilled in the art would lead that individual to combine the relevant teachings of the references. *In re Fine*, 5 USPQ2d 1596 (Fed. Cir. 1988). To invalidate claimed subject matter for obviousness, the combined teachings of the prior art references must suggest, expressly or by implication, the improvements embodied by the invention. *In re GPAC, Inc.*, 35 USPQ2d 1116 (Fed. Cir. 1995) (citing *In re Sernaker*, 702 F.2d 989, 217 USPQ 1 (Fed. Cir. 1983)). The teachings of references can be combined only if there is some suggestion or incentive to do so. *In re Fine*, supra.

This requirement goes to the question of motivation, and refers to a well established holding from earlier case law that there must be some logical reason at the time of the invention for combining the references along the lines of the invention; otherwise the use of the teachings as evidence of non-obviousness will entail prohibited hindsight. *Ex parte Stauber and Eberle*, 208 U.S.P.Q. 945, 946 (Bd. App. 1980).

As a further elaboration upon the above, the claims on appeal are composition claims and, by necessity, the patentability of these claims are evaluated by whether these compositions are *prima facie* obvious over prior art compositions taking into account issues such as the similarity of these compositions to the prior art. *In re Deuel*, 34 USPQ2d 1210 (Fed. Cir. 1995).

Based on the above criteria, Appellants maintain that these cited prior art references, either alone or in combination, fail to achieve a *prima facie* case of obviousness against their claimed compositions.

Specifically, the cited Sasaki articles are not pertinent to the now claimed invention because these articles fail to disclose resin/protein compositions where the resin, containing an ionizable functionality, is electrostatically uncharged at physiological pH's of from 5 to 9 while having protein bound thereto. Sasaki's teachings clearly lead to the conclusion that the ionizable Amberlite CG-50 resins employed therein are charged at any pH within this range because Sasaki states that a pH of 4.5 or less is required to completely protonate the carboxyl groups of the resins. Accordingly, at a pH of 5 or more, Sasaki's Amberlite CG-50 resins would carry an anionic charge. Sasaki himself recognized this limitation at page 1561 of his 1982 article where he states that:

> "However, hydrophobic-ionic chromatography with Amberlite CG-50 has the disadvantage that a pH as acidic as 4.5 is required in the process of adsorption".

Moreover, neither Sasaki article suggests the use of any ionizable ligands which would be electrostatically uncharged at a physiological pH range of from 5 to 9 and bind proteins. This failure of the Sasaki articles to suggest such ligands is recognized in both the final Office Action and the Advisory Action which do not allege that either Sasaki article teaches such ligands. At best, the Advisory Action states that Sasaki (1982) discloses the possibility of absorbents carrying alkaline groups although, as described by Sasaki (1982), the "relationship to pH would be opposite". Such an opposite relationship suggests to the skilled artisan an alkaline pH as far removed from neutrality (pH 7) as the Amberlite resins described by Sasaki (1982). Since Sasaki's Amberlite resins are reported to dissociate at pH 4.5, opposite alkaline charges would dissociate at pH 9.5 which is also outside the range claimed by Appellants.[2]

Notwithstanding the statements in the Advisory Action to the contrary, the Sasaki (1982) article would not provide any motivation to the skilled artisan to employ ionizable functionalities which would dissociate at pHs of from 5 to 9 because Sasaki (1982) is suggesting only alkaline groups which would be opposite carboxyl groups and further because the Advisory Action fails to state why groups which dissociate at pH 5 to 9 as per this invention would be "opposite" such carboxyl groups.

The secondary references relied upon in this rejection fail to cure the deficiencies of the Sasaki articles because these secondary references each employ resins requiring electrostatic charge during protein binding.

Specifically, the Kasche reference shows binding of proteins to a resin which, under the conditions employed by Kasche, contain significant positive charge and, accordingly, Kasche cannot disclose the resin/protein complexes of this invention. Specifically, Kasche's Figure 2 illustrates the charge density of phenylbutylamine (PBA)

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[2] The acidic pH of 4.5 is 2.5 units removed from a neutral pH of 7 and, accordingly, a basic pH equally removed from neutrality would require a pH of 9.5.

Eupergit resin from about pH 2-10 in the absence of bound protein. As shown in this figure, the resin contains substantial electrostatic charge at pH's of about 8 or less. However, Kasche's experimental section recites in the second and third paragraphs of page 151 and in Figure 3 that enzyme (e.g., penicillin amidase from *E. coli* homogenates) was contacted with the PBA-Eupergit resin at pH 7.5 and Kasche's Figure 1 recites a contacting the resin to an enzyme containing solution at pH 7. In each case, the PBA-Eupergit resin carries a significant electrostatic charge. In point of fact, Kasche's statement in the bridging sentence between pages 152 and 153 that:

> "...hydrophobic interactions cause the adsorption, and ... charge-charge repulsions on the support limit the adsorption capacity"

supports Kasche's recognition that his supports carry charge at the pH of absorption. Significantly, Kasche does not suggest that one should avoid this charge by adjusting the pH to a value where the resin is electrostatically uncharged.

As to the Teichberg and Jost references, these are irrelevant to the claims in this application because neither reference is addressing the problem solved by this invention.

Specifically, Teichberg is concerned with affinity-repulsion chromatography whereas Appellants' methods are not directed to affinity chromatography. Further, and more to the point, Teichberg recites at page 54 et seq. that the matrices employed therein are charged when the protein is bound to the resin. Such a requirement is contrary to Appellants' claimed invention.

Jost is concerned with determining whether binding of negatively charged proteins such as ovalbumin and β-lactoglobulin is due to hydrophobic or electrostatic interactions. In any event and, again, more to the point, Jost similarly discloses the necessity of charged groups (i.e., positively charged groups) in his resins to effect protein recovery.

Specifically, Jost compares resins conventionally charged at physiological pH (i.e., CNBr activated agarose derivatized with alkyl- and arylamines) versus resins which apparently are uncharged at physiological pH but having one or two dissociation ranges (agarose derivatized with alkyl or aryl hydrazides). Jost states at page 75 (column 2) that:

> "The experiments presented in this paper describe the adsorption of ovalbumin, α-lactalbumin, and leucine aminopeptidase (EC 3.4.1.1) to alkyl- and arylamino-agaroses and demonstrate the abolishment of such binding in structurally closely related uncharged agarose derivatives, prepared from the corresponding alkyl or aryl hydrazides."

The only protein which bound to the uncharged agarose derivatives of Jost was bovine serum albumin (BSA) which Jost recites as being "bound almost irreversibly" to this resin. In point of fact, Jost describes that attempted "[d]esorption [of the BSA] with 1 M NaCl was unsuccessful". See, for example, the first five lines under Table I of Jost. However, Jost describes that the use of positively charged resin permits binding and recovery of BSA. See, e.g., Table 1 of Jost. Accordingly, Jost teaches that in the absence of charged groups on the resin, two proteins did not bind to the resin and a third (BSA) bound apparently irreversibly to the resin thus preventing recovery of the BSA. As is apparent, irreversible binding in this third resin does not lend itself to protein recovery from an aqueous mixture.

In view of the above, each of the cited secondary references fails to teach the desirability of employing an ionizable but electrostatically uncharged resin in the physiological pH range of 5 to 9 during protein recovery and, hence, cannot cure the deficiencies of Sasaki ('79 and/or '82).

Accordingly, the combination of references provided in this rejection simply fails to suggest to one skilled in the art the desirability of making the compositions of this invention. Nor do these references, either alone or in combination, suggest these

compositins or provide a reasonable expectation of success to a skilled artisan that the modifications necessary to either Sasaki article to arrive at the claimed invention would be successful in effecting protein recovery. Absent such suggestion and reasonable expectation, this rejection is in error. *In re Vaeck, supra.*

As to the new/additional arguments raised in the Advisory Action regarding the cited secondary references, such arguments are simply not germane to the issue presented herein. Specifically, the Advisory Action alleges that notwithstanding the partial charge on the resin/protein complexes described in these secondary references, these resins are somehow germane to the claims on appeal.[3] The Advisory Action also alleges that Sasaki (presumably Sasaki 1982) predicts that partially charged resin can still bind protein and, accordingly, the teachings of the prior art regarding partial charge is again somehow germane to the appealed claims.

These new/additional arguments fail, however, to account for the fact that the appealed claims are *composition* claims and, hence, patentability is premised upon the issue of motivation to prepare these compositions with a reasonable expectation of success. *In re Vaeck, supra.* For the sake of clarity, such compositions are resin/protein complexes wherein the resin is characterized as being electrostatically uncharged at the pH of protein absorption. The Advisory Action, on the other hand, recognizes that these secondary references fail to disclose such resins but relies upon Kasche's partial charge at the pH of binding to allege the requisite motivation. Appellants' claims do not, however, recite that the resin is partially charged at the pH of protein binding and, accordingly, such reliance is misplaced.

As to the allegation in the Advisory Action that Sasaki et al. predicts that a protein can still bind to the resin which is partially charged, Appellants again maintain that such

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[3] See, for example, the arguments raised in the Advisory Action regarding the Kasche, et al. article.

an allegation is not germane to the appealed claims which do not recite binding to a partially charged resin.

Additionally, Appellants are unable to find such an allegation/prediction recited in Sasaki, et al. Rather, this allegation is merely the Examiner's interpretation of Sasaki's cartoon. Such an interpretation is, however, inconsistent with the teachings of Sasaki, et al. because this article does not recite this alleged prediction and further because the data provided by Sasaki, et al. is inconsistent therewith. Specifically, Sasaki (1982) recites at page 1557[4] that the protein glucose oxidase from *Aspergillus niger* was bound to the Amberlite resin at pH 4.5 and was eluted from this resin at a pH centered at 4.8. If as Sasaki alleges, the resin is uncharged at pH 4.5, the adjustment to pH 4.8 would involve only partial ionization of the resin. Accordingly, these results are inconsistent with the Examiner's interpretation of Sasaki, et al.'s cartoon because this article specifically discloses desorption under conditions wherein the resin is only partially charged.

Rather, Appellants maintain that Sasaki, et al.'s cartoon is only illustrative in nature to show that incorporation of negative charges, including only partial negative charges, onto the resin results in separation of the protein from the resin. That is to say that the left hand portion of the cartoon is illustrative to indicate that upon presentation of charged groups on the resin, these charged groups interact with similar charged groups on the protein to force the protein off the resin. Such desorption is illustrated in the right hand portion of the cartoon.

In view of the above, Appellants maintain that the further arguments raised in the Advisory Action are not germane to the patentability of the appealed claims.

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[4] The relevant disclosure in Sasaki (1982) is found at page 1557 immediately after the caption "RESULTS".

IX. Conclusion

In view of the above, Appellants respectfully submit that the rejection of Claims 1-5 and 7-23 is in error and, accordingly, request that the Board of Patent Appeals and Interferences reverse this rejection of Claims 1-5 and 7-23 as set forth above.

An early decision on the merits is earnestly solicited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: Gerald F. Swiss

Gerald F. Swiss
Registration No. 30,113

by. Leslie A. Mooi
Leslie A. Mooi
Reg. No. 37,047

P.O. Box 1404
Alexandria, VA 22313-1404
(415) 854-7400

Date: August 19, 1997

APPENDIX I

The Appealed Claims

1. A resin-protein/peptide complex which comprises a resin and a target protein or peptide bound thereto wherein said resin comprises

a) a solid support matrix; and

b) selected ionizable ligand covalently attached to the matrix

wherein the ionizable ligand is selected such that the resin is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin wherein the protein or peptide binds to the resin at a pH of 5 to 9 and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin wherein desorption occurs by a change in the pH from the binding pH and further wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or low ionic strength.

2. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is positively charged at the pH where the target protein or peptide is desorbed from the resin.

3. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is negatively charged at the pH where the target protein or peptide is desorbed from the resin.

4. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand comprises an ionizable functional group directly attached to the solid support matrix.

5. The resin-protein/peptide complex of Claim 1 wherein the ionizable ligand comprises a spacer arm and at least one ionizable functionality wherein the ionizable functionality is attached to the solid support matrix via the spacer arm.

7. The resin-protein/peptide complex of Claim 1 wherein the resin further comprises non-ionizable ligands.

8. The resin-protein/peptide complex of Claim 7 wherein the percentage of non-ionizable ligands attached to the solid support matrix based on the total of ionizable and non-ionizable ligands ranges from greater that 0% to about 80%.

9. The resin-protein/peptide complex of Claim 8 wherein the percentage of non-ionizable ligands attached to the solid support matrix based on the total of ionizable and non-ionizable ligands ranges from greater than 0% to about 40%.

10. The resin-protein/peptide complex of Claim 1 wherein the solid support matrix is cross-linked.

11. The resin-protein/peptide complex of Claim 1 wherein the resin contains from about 0.05 mmol to about 0.5 mmol ionizable ligand per ml of the solid support matrix prior to covalent attachment of any non-ionizable ligand.

12. The resin-protein/peptide complex of Claim 1 wherein the solid support matrix is non-ionizable.

13. The resin-protein/peptide complex of Claim 1 wherein the solid support matrix contains ionizable functionality which functionality is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin.

14. The resin-protein/peptide complex of Claim 1 wherein the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the same polarity as the net electrostatic charge on the target protein or peptide at the pH of desorption.

15. The resin-protein/peptide complex of Claim 1 wherein the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the opposite polarity from the net electrostatic charge on the target protein or peptide at the pH of desorption.

16. A resin-protein/peptide complex which comprises a resin and a target protein or peptide bound thereto wherein said resin comprises

a) a solid support matrix having a selected ionizable functionality incorporated into the backbone thereof wherein the ionizable functionality is selected such that the resin is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin wherein the protein or peptide binds to the resin at a pH of 5 to 9 and is electrostatically charged at the pH where the target protein or peptide is desorbed from the resin wherein desorption occurs by a change in the pH from the binding pH; and

b) optionally a non-ionizable ligand covalently attached thereto,

wherein about 50 percent or more of the target protein or peptide in an aqueous medium binds to the resin when the aqueous medium has either a high or low ionic strength.

17. The resin-protein/peptide complex of Claim 16 wherein the ionizable functionality is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is positively charged at the pH where the target protein or peptide is desorbed from the resin.

18. The resin-protein/peptide complex of Claim 16 wherein the ionizable functionality is electrostatically uncharged at the pH where the target protein or peptide is bound to the resin and is negatively charged at the pH where the target protein or peptide is desorbed from the resin.

19. The resin-protein/peptide complex of Claim 16 wherein the ionizable functionality comprises amino groups covalently attached in the backbone of the solid support matrix.

20. The resin-protein/peptide complex of Claim 16 wherein the solid support matrix is cross-linked.

21. The resin-protein/peptide complex of Claim 16 wherein the resin contains from about 0.05 mmol to about 0.5 mmol non-ionizable ligand per ml of the solid support matrix.

22. The resin-protein/peptide complex of Claim 16 wherein the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the same polarity as the net electrostatic charge on the target protein or peptide at the pH of desorption.

23. The resin-protein/peptide complex of Claim 16 wherein the electrostatic charge induced on the resin of the resin-protein/peptide complex is of the opposite polarity from the net electrostatic charge on the target protein or peptide at the pH of desorption.

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